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Poster Presentation



# Caspase activation, sialidase release and changes in sialylation pattern of recombinant human erythropoietin produced by CHO cells in batch and fed-batch cultures

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Product yield and product sialylation are the two most principal yardsticks that determine the commercial viability of most recombinant cell culture processes. In this study, we describe a strategy whereby an optimized harvesting time point was established based on product titer and sialylation pattern of recombinant human erythropoietin (EPO) produced in a CHO cell line. The activation of various caspases, the release of intracellular sialidase and the changes in sialylation pattern of the recombinant product in medium were tracked in both batch and fed-batch cultures. In both setups, all caspase activities were found to peak at the culture time point at which declivity of cell viability was most pronounced. Also, release of intracellular sialidase and lactate dehydrogenase (LDH) coincided with the observed decline in cell viability and the concomitant increase in caspase activities. By incorporating isoelectric focusing (IEF), coupled with double blotting as the de novo technique in the analysis of product sialylation, prompt resolution of secreted EPO isoforms in a time course format were obtained. The IEF profile of the batch culture showed relatively good consistency in product sialylation compared to fed-batch culture, which showed gradual band shifts towards more basic isoforms as the culture progressed. Based on key parameters such as product yield and extent of product sialylation, the optimized harvesting time point was found to be at the 91 % culture viability mark in both batch and fed-batch cultures. In addition, various metabolite analyses were conducted in a bid to identify potential death inducing factors present in batch and fed-batch cultures. Accumulation of metabolites, coupled with elevated osmolality in fed-batch culture was found to be sufficient in its propensity to impede cell growth and to moderately induce cell death.